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Disentanglement of Heterogeneous Dynamics in Mixed Lipid Systems

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ABSTRACT Static phosphorous NMR has been a powerful technique for the study of model supramolecular phospholipid structures. Application to natural lipid bilayers with complex compositions, however, has been severely limited by the difficulty in deconvoluting overlapping broad lineshapes. We demonstrate a solution to this problem, using a global fit to a few slow magic-angle spinning spectra, in combination with an adaptation of Boltzmann statistics maximum entropy. The method provides a model-free means to characterize a heterogeneous mix of lipid dynamics via a distribution of ^{31}P chemical shift anisotropies. It is used here to identify clear changes in membrane dynamics of a phosphatidylethanolamine and phosphatidylglycerol mixture, mimicking an *Escherichia coli* membrane upon addition of just 2% of the antimicrobial peptide maculatin 1.1. This illustration opens the prospect for investigation of arbitrarily complex natural lipid systems, important in many areas of biophysical chemistry and biomedicine.

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With an escalation of interest in the role of lipids in natural membranes, there has been a resurgence in use of phosphorus NMR for the characterization of phospholipid systems. Disruption of bilayer order and dynamics by physiologically active molecules such as antimicrobial peptides are of particular recent interest, as these represent a potential new class of antibiotics (1). Membrane lipid compositions are highly variable in polar headgroups, backbones, and acyl chains—in contrast to the very simple lipid systems typically used to mimic them. This simplicity is motivated in part by the difficulty of interpreting spectra with overlapping ^{31}P static lineshapes arising from many local motional differences. More complex lipid systems that better mimic natural membranes can usually only be analyzed in terms of overall width, and other differences are discussed only qualitatively.

In this Letter we demonstrate a means to characterize a heterogeneous distribution of chemical shift parameters in ^{31}P NMR spectra of mixed lipid vesicles. The approach uses just a few simple one-dimensional magic-angle spinning spectra (MAS) at slow spin rates, which can be collected with sufficient signal/noise in less time than a static spectrum. MAS also mitigates the complexities of lifetime broadening and magnetic field-induced lipid alignment (2) that plague interpretation of static spectra.

The method also obviates the difficult choice of any specific number of components to deconvolute a static spectrum, by employing a Boltzmann-type maximum entropy strategy that gives a pseudo-continuous distribution of chemical shift anisotropy (δ) and asymmetry (η) parameters. The analysis is performed with the use of Lagrange multipliers, introduced in an analogous fashion to the usual derivation of the Boltzmann distribution, as demonstrated previously for the analysis of rotational-echo double-resonance data (3).

Spinning of the semisolid lipid vesicle sample around the magic-angle of

$$\arccos\left(\sqrt{1/3}\right) = 54.74^{\circ}$$

modulates the otherwise broad static NMR signal into a manifold of peaks separated by the spinning rate (4). The capacity to analyze the relative spinning sideband intensities to recover chemical shift parameters encoded in them has expanded over the last three decades, in parallel with advances in computational resources (5–11). For heterogeneous samples, where a distribution of chemical shift anisotropies and asymmetries may arise from different lipid domains and local phase differences, the problem may be represented as

$$\vec{\mathcal{I}}_{(N,\nu_r)} = \underset{\approx}{\mathcal{I}}\,\vec{p}_{(\delta,\eta)},$$

where $\vec{p}_{(\delta,\eta)}$ is a linearized representation of the two-dimensional distribution over chemical shift parameters (δ,η); $\vec{\mathcal{I}}_{(N,\nu_r)}$ is the series of experimental sideband intensities N over one or more spinning speeds (ν_r), each normalized to unity over N; and $\underset{\approx}{\mathcal{I}}$ is the matrix of precalculated intensities (5), for distribution parameters $(\delta,\eta)_i$ and data parameters $(N,\nu_r)_j$,

$$\mathcal{I}_{i,j} = \frac{1}{4\pi^2}\int_0^{2\pi} d\alpha \int_0^{\pi} P(\beta) d\beta \frac{1}{4\pi^2}\left\{\left[\int_0^{2\pi} d\gamma \cos(\Phi')\right]^2 + \left[\int_0^{2\pi} d\gamma \sin(\Phi')\right]^2\right\},$$

where the probability $P(\beta) \propto \sin(\beta)$ as usual (4,5), and Φ' gives the unweighted and unnormalized contribution to sideband N_j at MAS rate ν_{rj} by a crystallite with chemical-shift parameters δ and η, and at an orientation (α, β, γ) with respect to B_0,

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$$\Phi' = N_j\gamma + \frac{\delta_i\eta_i}{\nu_{r_j}}\Phi_A - \frac{\delta_i}{\nu_{r_j}}\Phi_B,$$

with

$$\Phi_A = \frac{1}{24}\cos 2\alpha(3+\cos 2\beta)\sin 2\gamma + \frac{\sqrt{2}}{6}\cos 2\alpha \sin 2\beta \sin\gamma + \frac{\sqrt{2}}{3}\sin 2\alpha \sin\beta \cos\gamma + \frac{1}{6}\sin 2\alpha \cos\beta \cos 2\gamma$$

$$\Phi_B = \frac{1}{8}(1-\cos 2\beta)\sin 2\gamma - \frac{1}{\sqrt{2}}\sin 2\beta \sin\gamma.$$

Using custom code written in C/C++ with MPI (http://www.lam-mpi.org/mpi/) to parallelize matrix element calculation over 16 2.3 GHz AMD Opteron 2376 processors (Advanced Micro Devices, Sunnyvale, CA), calculation of ~10^6 crystallite orientations, 34–36 sidebands (over three speeds), with 281 δ and 21 η combinations took ~45 min.

Much of the earliest work in lipid structure and dynamics used lecithin, in which phosphatidylcholine (PC) lipids dominate. Subsequent work using synthetic lipids continued to employ PC predominantly, but more recent work is exploring the use of other lipids to better represent key characteristics such as negative surface charges (12–14). Bacteria, however, tend to have no diacyl PC; phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin tend to dominate instead, and proportions of these components vary widely over different bacterial species (15).

Here we demonstrate a solution to difficulties encountered in studying even the relatively simple mixture of 70% DMPE/30% DMPG (16), which better approximates *Escherichia coli* outer membranes than generic PE-dominated mixtures. With a view to understanding the antimicrobial action of the maculatin 1.1 peptide and its potential as an antibiotic lead, we conduct the study at physiological temperature (37°C). This temperature is intermediate between the gel-to-fluid phase transition temperatures for pure DMPE (50°C) and pure DMPG (24°C). Lipids in this mixture may be resident in variously enriched domains, and exchanging between them. The complex structure and phase characteristics of the lipid-only system can only be exacerbated when a peptide is added to the system. The static ^{31}P NMR spectra of these systems (Fig. 1) thus feature a number of overlapped broad line spectral components with nearly degenerate chemical shift. Fluid phase lipids undergo rapid motions that produce a reduced symmetry ($\eta = 0$) static lineshape, and gel phase lipids are not motionally averaged by long axis lipid rotation, and produce a broad asymmetric line. With additional parameters needed to account for the broad linewidths inherent in the short signal lifetime, all attempts to deconvolute the static spectra into a small number (≤ 3) of components proved unsatisfactory.

By MAS, here at 1.2, 1.5, and 1.8 kHz in a 14.1 T magnetic field (Figs. 1 and 2), the same chemical-shift information is encoded into three sets of spinning sidebands. In some cases, chemical shifts that are characteristic of different lipid environments can be resolved from each other by spinning (17), and each interleaved set of sidebands may correspond to a single dynamical mode. More typically, resolution is insufficient, and spectral components with different chemical-shift parameters will add to each sideband intensity at different speeds, but in a predictable way (Fig. 2). The Boltzmann statistics analysis begins with a flat distribution, and results in a distribution of chemical-shift anisotropies that are not specifically excluded by the data (Fig. 3).

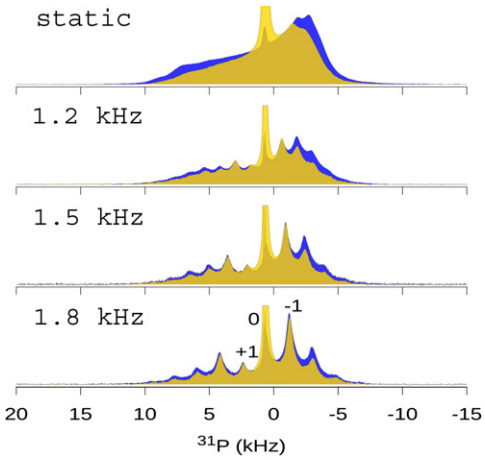

FIGURE 1 Static and MAS 80 kHz SPINAL-64 ^{1}H-decoupled spectra for 70% DMPE/30% DMPG (*blue*) and the same lipid system with 2% (mol/mol) maculatin 1.1 added (*gold*). MAS spectra are scaled to match the −1 spinning sidebands to ease comparison. Opacity is reduced to help discern where spectra overlap.

In the peptide-free 70% DMPE/30% DMPG lipid system, we find a range of anisotropies centered at $\delta \simeq 8$ kHz, and slightly asymmetric with $\eta \simeq 0.1$–0.2. With the addition of 2% maculatin, aside from the obvious increase in isotropic component (fit explicitly during deconvolution, then excluded from the analysis), we detect the possibility of a more disordered population at $\delta \simeq 5$ kHz, and a decreased asymmetry of the $\delta \simeq 8$ kHz component. These changes are consistent with a peptide-induced lowering of the bulk lipid-phase transition temperature, or a change in lipid mixing characteristics.

Detection of these subtle potential lipid environment differences in lipid mixtures by a small amount of antimicrobial peptide undergoing complex dynamics required just a few hours for NMR acquisition, spectral processing, and maximum entropy analysis. It is worth noting that other sample compositions and conditions are often even easier to analyze, with more favorable relaxation times that allow sufficient sideband resolution at slower spinning speeds.

The analysis on the data shown attests to the robustness and efficiency of the method to identify different lipid components or domains in mixed lipid bilayers, and expands our capacity to understand complex lipid systems. To our knowledge, such information has not been accessible using standard experiments. Further benefits of this approach include the liberation of the spectroscopist from troublesome

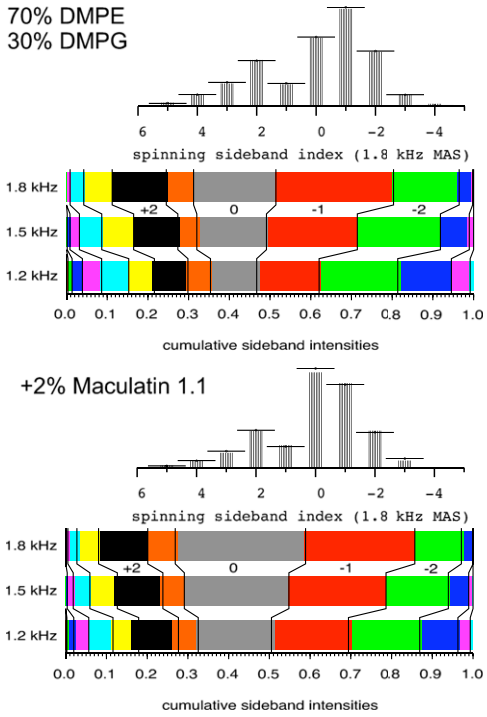

FIGURE 2 The upper, monochrome portion of each panel shows experimental sideband intensities (*horizontal bars*) and eight independent fits (*vertical impulses*) for Boltzmann statistics maximum entropy fits at 1800 Hz MAS rate, for lipid-only and lipid-plus-peptide. Sideband intensity error estimates are negligible. The lower, colored portion of each panel shows normalized sideband intensities (totaling unity) for one of the fits at each spinning speed as horizontal colored histograms relative to experimental sideband intensities (*black lines*). The evolution of fractional signal intensities over the sideband indices at different spin rates provides a sense of the information content of the data.

decision-making during deconvolution analysis, and that the prospect of studying membrane-interacting agents with natural, intact membranes becomes more attainable.

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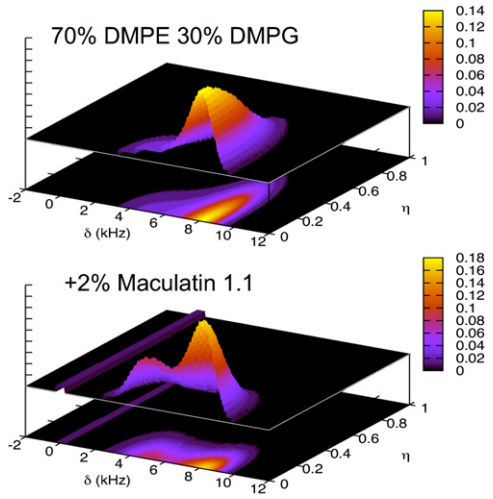

FIGURE 3 One of the eight essentially identical distributions resulting from the Boltzmann maximum entropy analysis for 70% DMPE/30% DMPG control and for lipid plus 2% Maculatin 1.1. The peptide appears to induce a component with $\delta = 5$ kHz, corresponding to an axially symmetric species with full linewidth $\Delta\delta = \delta_{\perp} - \Delta\delta_{\parallel} = -30.8$ ppm, and reduces the asymmetry of the $\delta = 8.1$ kHz component, corresponding to another axially symmetric lineshape of $\Delta\delta = -50$ ppm.

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